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Damage to histaminergic tuberomammillary neurons and other hypothalamic neurons with traumatic brain injury

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Damage to histaminergic tuberomammillary neurons and other hypothalamic neurons with traumatic brain injury

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* Equal contribution

Running head: Loss of histamine neurons with traumatic brain injury

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Abstract

The need for increased sleep after traumatic brain injury is a common and disabling complaint, yet its etiology is unknown. Previous studies have demonstrated diffuse damage to various hypothalamic systems, but the integrity of the histaminergic tuberomammillary nucleus, a major arousal-promoting system located in the posterior hypothalamus, has never been examined in head trauma patients. Here, we demonstrate that severe head trauma is associated with a marked loss (41%) of histaminergic neurons. Reduced histamine signaling may contribute to increased sleep need, and therapies that enhance histaminergic tone may improve arousal after head trauma or other conditions.

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Introduction

Traumatic brain injury (TBI) is the leading cause of mortality and disability among young individuals in high-income countries,¹ and the incidence has steadily increased over the last decade.² As advances in the treatment of TBI have improved survival³, management of the chronic consequences of TBI has become a growing medical challenge.

Among these chronic sequelae, sleep-wake disorders are especially common, particularly disturbances of arousal such as excessive daytime sleepiness and pleiosomnia (increased sleep need per 24 hours).^{4,5} Posttraumatic insomnia also can occur, although a prospective study using polysomnography and actigraphy reported a prevalence of only 5%.⁴ Sleep-wake disturbances occur in 30-40% of TBI patients, but therapies to improve arousal after TBI are very limited.⁶

Surprisingly little is known about injury to the hypothalamus and other wake-promoting brain regions in TBI, but in a pilot study of 4 TBI patients and 4 controls without head injury, we found a 27% loss of wake-promoting orexin (hypocretin) neurons.⁷ To improve our understanding of injury to wake-promoting circuitry in TBI, we have now examined the hypothalamus after TBI in a larger number of subjects, with an additional focus on histaminergic neurons of the tuberomammillary nucleus (TMN).

Materials and Methods

Human subjects. We obtained brains from 12 subjects with TBI from the Neuropathology Departments of Graz and Linz, Austria. Since trauma-induced pathological changes may not be apparent in the acute period after head trauma, we included only patients who survived at least 7 days after TBI. We examined hypothalamic tissue from the optic chiasm rostrally, back through several millimeters caudal to the posterior edge of the mammillary body. In addition, we studied hypothalami from 16 control subjects without TBI or other neurological disease, provided by the Neuropathology Departments of Beth Israel Deaconess Medical Center (n=10) and Graz, Austria (n=6). Controls died from myocardial

infarction or acute heart failure (n=7), pneumonia (n=2), acute renal failure (n=2), pulmonary embolism (n=1), upper gastrointestinal bleeding (n=1), colon perforation (n=1), pericarditis (n=1) and acute pancreatitis (n=1). Patients or controls with a history of previous TBI, known sleep-wake disturbances or evidence of neurological or psychiatric disease prior to TBI were not considered for this study. The local ethics committees of all involved institutes approved the study protocol.

Brain tissue processing and immunohistochemistry. All hypothalami were fixed in 10% buffered formalin and stored at 4°C. The mean fixation times of TBI and control brains were similar and varied from 1 month to 2 years. Immunohistochemical studies were focused on hypothalamic systems involved in the regulation of sleep and wake, including the neurons producing histamine, melanin-concentrating hormone (MCH) and orexin A. We identified histamine-producing neurons by immunostaining for histidine decarboxylase (HDC), the main enzyme responsible for the production of histamine. We used methods identical to those recently described.⁸

To examine the pattern and severity of neuronal damage, axonal injury and gliosis within the hypothalamus, we used hematoxylin and eosin (H&E) and glial fibrillary acidic protein (GFAP) staining. To quantify gliosis, we measured the density of GFAP-immunoreactive astrocytes (soma) as described in **Fig. 2**.

Stereological cell counts. We used the same stereological methods as previously described.⁸

Statistics. Group data are reported as means and standard deviations (SD). We established that data were normally distributed using the Kolmogorov-Smirnov test. Therefore, we compared cell counts between groups using Student's t test and performed correlation analyses using the Pearson test. To assess whether the severity of cell loss differentially affected the examined neuronal populations, we applied ANOVA statistics followed by Bonferroni correction tests. Statistical significance was accepted at $p < 0.05$.

Results

Clinical characteristics. The 12 TBI patients and the 16 controls did not differ by age (70 ± 13 vs. 68 ± 12 years, $p=0.64$) or gender (male/female ratio: 9/3 vs. 9/7, $p=0.27$).

Postmortem delay (PMD; the interval between death and autopsy) tended to be longer in the TBI group (29.8 ± 26.2 vs. 19.1 ± 7.3 hours, $p=0.19$), but PMD did not correlate with the numbers of HDC, MCH or orexin A neurons. The TBI patients had closed head injury from traffic accidents ($n=5$) or falls ($n=7$), were comatose at hospital admission, and died in the intensive care unit with a mean survival after trauma of 27 ± 28 days (range: 7–85 days). We did not find any correlation between duration of TBI survival and cell counts of HDC ($r=-0.61$, $p=0.14$), MCH ($r=-0.16$, $p=0.73$) and orexin ($r=-0.13$, $p=0.79$).

General neuropathological findings. H&E staining did not reveal any abnormalities in the hypothalamus of all 16 controls and in 5 TBI patients. The remaining 7 TBI patients had a variety of findings, including acute hemorrhage, microglial nodules, rarefaction of neuropil, macrophage infiltration near disrupted white matter tracts, infarct, and axonal swellings (**Fig. 1**). Three TBI patients had minor acute hemorrhages in the lateral hypothalamic area, lateral tuberomammillary nucleus, medial forebrain bundle, or cerebral peduncle as well as nearby regions including the putamen, globus pallidus, nucleus basalis of Meynert, or ventral anterior thalamic nucleus. The other four TBI patients showed more chronic patterns of injury with axonal swellings, loss of neuropil, and microglial nodules. Overall, the neuropathological abnormalities tended to be more prominent in the posterior than in the anterior part of the hypothalamus. The density of activated astrocytes indicating TBI-induced reactive gliosis, as assessed by GFAP immunostaining, was most pronounced around the border of the third ventricle. In this region, we observed gliosis even in hypothalamic tissue of controls, but in TBI patients, the density of activated astrocytes was higher and extended further into the hypothalamus than in controls (**Fig. 2**).

Loss of specific hypothalamic neuronal populations. The number of histaminergic neurons in TBI patients was reduced by 41% compared to controls ($71,837 \pm 8,787$ vs. $122,290 \pm 12,064$, $p < 0.001$) (**Fig. 3, 4**). Asymmetry of HDC cell counts between left and right hypothalami was significantly higher in TBI patients than in controls ($11.7 \pm 6.8\%$ vs. $4.5 \pm 3.3\%$, $p = 0.008$), suggesting focal and asymmetric injury. In the TBI group, left-right asymmetry was positively correlated with more severe HDC cell loss ($r = -0.68$, $p = 0.03$). In addition, loss of histaminergic neurons in TBI patients was significantly more pronounced in the posterior half of the TMN compared to the anterior half ($48.8 \pm 22.9\%$ vs. $32.3 \pm 21.7\%$, $p = 0.02$). Compared to controls, TBI patients also had 29% fewer MCH neurons ($58,232 \pm 8,944$ vs. $82,054 \pm 12,834$, $p < 0.001$) and 21% fewer orexin A neurons ($49,830 \pm 9,851$ vs. $62,935 \pm 8,704$, $p = 0.001$). The HDC neuron loss was significantly greater than the MCH and orexin A neuron loss ($p = 0.048$ and $p < 0.001$, respectively), whereas the 29% MCH and 21% orexin A neuron loss was of similar magnitude ($p = 0.29$). There was no significant left-right asymmetry of MCH and orexin-A neurons in either group, and loss of MCH and orexin A neurons in TBI patients did not show any significant rostro-caudal difference.

Discussion

Excessive sleepiness and pleiosomnia are common after TBI, and we found a 41% loss of the wake-promoting histaminergic neurons in the TMN of patients with severe TBI. We also found moderate loss of the MCH (29%) and orexin A (21%) neurons as well as evidence of small hemorrhages, axonal injury, and gliosis in the hypothalamus. These findings demonstrate that severe TBI often injures the hypothalamus, and loss of specific wake-promoting neurons may contribute to increased sleep need.

The observed pattern of cell loss suggests that with severe TBI, hypothalamic injury may be greatest in the most posterior parts. Of the examined neuronal populations, the TMN neurons showed the greatest cell loss, especially in the caudal half of the nucleus. The TMN is in the most posterior part of the hypothalamus, and it may be more susceptible to shearing

injury at the hypothalamus-midbrain junction. In contrast, the MCH and orexin A populations revealed less injury, perhaps because they are slightly further from the ventral surface of the hypothalamus.

Histamine is a potent wake-promoting neurotransmitter, and loss of the histaminergic neurons may contribute to excessive daytime sleepiness and pleiosomnia after TBI. TMN neurons are mainly active during arousal and sustained wakefulness⁹⁻¹¹, and their wake-maintaining effect is clear from the sedating effects of centrally-acting antihistamines.¹² In addition, mice lacking histidine decarboxylase have less wakefulness at the beginning of their active period and sleep more in a novel environment.¹³ The marked increase in histaminergic neurons in narcolepsy^{8,14} may be a compensatory response to the near-total loss of arousal-promoting orexin neurons, and further emphasizes the importance of the histamine system in maintaining normal wakefulness. Pitolisant, a selective inverse agonist of the histamine H₃ receptor, increases histamine signaling and improves sleepiness in narcolepsy patients.¹⁵ Thus, loss of histaminergic neurons in TBI may contribute to posttraumatic pleiosomnia, and increased sleep need may respond well to medications that enhance histamine signaling.

The clinical significance of the observed 21% loss of hypocretin neurons remains unclear. In general, it is assumed that neurotransmitter signaling falls only with extensive cell loss, but a recent study shows that even a 14-23% reduction of nigral dopaminergic neurons suffices to cause mild Parkinsonism.¹⁶ Thus, the modest loss of orexin A neurons may also contribute to sleepiness, and could be another good therapeutic target once methods to improve hypocretin signaling become available.

Interpreting the functional impact of the 29% loss of MCH neurons is more of a challenge as much less is known about the normal functions of this system. The MCH neurons probably promote sleep as they are active during REM sleep¹⁷, fire maximally during sleep¹⁸, and activation of these neurons increases both non-REM and REM sleep^{19, 20}. Thus, it can be speculated that deficiency in MCH signaling potentially impairs recovery from TBI

by reducing sleep consolidation, but these effects were masked in our TBI population by their coma.

A limitation of our study is the inclusion of only patients with severe TBI that resulted in persistent coma. Thus, we cannot comment on specific sleep disorders in these patients, and the loss of hypothalamic neurons may be more extreme than in patients with less severe head trauma. Also, factors linked to prolonged coma, secondary complications and differences in treatment prior to death could impact the observed neuropathology. Though it will be challenging, it would be ideal if future clinical-anatomical studies examined the brains of TBI patients with lengthy survival and well documented sleep-wake disturbances.

In conclusion, we found that severe TBI substantially injures the histaminergic TMN neurons and moderately injures the orexin A and MCH neurons. Conventional H&E examination of the hypothalami from TBI patients showed acute and chronic lesions in several cases, but even those lacking H&E changes had loss of these neuropeptide-producing neurons, suggesting that the extent of hypothalamic injury after TBI may be underestimated using conventional approaches. The discovery of injury to the TMN neurons may have important clinical and therapeutic implications for TBI and perhaps other conditions with increased sleep need.

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References

1. Maas AIR, Stocchetti N, Bullock R. Moderate and severe traumatic brain injury in adults. *Lancet Neurol* 2008;7:728-741.
2. Frost RB, Farrer TJ, Primosch M, Hedges DW. Prevalence of Traumatic Brain Injury in the General Adult Population: A Meta-Analysis. *Neuroepidemiology*. 2012;40:154-159.
3. Stiefel MF, Spiotta A, Gracias VH, et al. Reduced mortality rate in patients with severe traumatic brain injury treated with brain tissue oxygen monitoring. *J Neurosurg* 2005;103:805-811.
4. Baumann CR, Werth E, Stocker R, Ludwig S, Bassetti CL. Sleep-wake disturbances 6 months after traumatic brain injury: a prospective study. *Brain* 2007;130:1873-1883.
5. Sommerauer M, Valko PO, Werth E, Baumann CR. Excessive sleep need following traumatic brain injury: a case-control study of 36 patients. *J Sleep Res* 2013;22:634-639.
6. Kaiser PR, Valko PO, Werth E, Thomann J, Meier J, Stocker R, Bassetti CL, Baumann CR. Modafinil ameliorates excessive daytime sleepiness after traumatic brain injury. *Neurology* 2010;75:1780-1785.
7. Baumann CR, Bassetti CL, Valko PO, et al. Loss of hypocretin (orexin) neurons with traumatic brain injury. *Ann Neurol* 2009;66:555-559.
8. Valko PO, Gavrillov YV, Yamamoto M, et al. Increase of histaminergic tuberomammillary neurons in narcolepsy. *Ann Neurol* 2013;74:794-804.
9. Steininger TL, Alam MN, Gong H, Szymusiak R, McGinty D. Sleep-waking discharge of neurons in the posterior lateral hypothalamus of the albino rat. *Brain Res* 1999;840:138-147.
10. Ko EM, Estabrooke IV, McCarthy M, Scammell TE. Wake-related activity of tuberomammillary neurons in rats. *Brain Res* 2003;992:220-226.

11. Takahashi K, Lin JS, Sakai K. Neuronal activity of histaminergic tuberomammillary neurons during wake-sleep states in the mouse. *J Neurosci* 2006;26:10292-10298.
12. Roehrs TA, Tietz EI, Zorick FJ, Roth T. Daytime sleepiness and antihistamines. *Sleep* 1984;7:137-141.
13. Parmentier R, Ohtsu H, Djebbara-Hannas Z, Valatx JL, Watanabe T, Lin JS. Anatomical, physiological, and pharmacological characteristics of histidine decarboxylase knock-out mice: evidence for the role of brain histamine in behavioral and sleep-wake control. *J Neurosci* 2002;22:7695-7711.
14. John J, Thannickal TC, McGregor R, et al. Greatly increased numbers of histamine cells in human narcolepsy with cataplexy. *Ann Neurol* 2013;74:786-793.
15. Dauvilliers Y, Bassetti C, Lammers GJ, et al. Pitolisant versus placebo or modafinil in patients with narcolepsy: a double-blind, randomised trial. *Lancet Neurol* 2013;12:1068-1075.
16. Tabbal SD, Tian L, Karimi M, Brown CA, Loftin SK, Perlmuter JS. Low nigrostriatal reserve for motor Parkinsonism in nonhuman primates. *Exp Neurol* 2012;237:355-362.
17. Luppi PH, Clément O, Fort P. Paradoxical (REM) sleep genesis by the brainstem is under hypothalamic control. *Curr Opin Neurobiol* 2013;14:714-718.
18. Monti JM, Torterolo P, Lagos P. Melanin-concentrating hormone control of sleep-wake behavior. *Sleep Med Rev* 2013;17:293-298.
19. Konadhode RR, Pelluru D, Blanco-Centurion C, et al. Optogenetic stimulation of MCH neurons increases sleep. *J Neurosci* 2013;33:10257-10263.
20. Jegu S, Glasgow SD, Herrera CG, et al. Optogenetic identification of a rapid eye movement sleep modulatory circuit in the hypothalamus. *Nat Neurosci* 2013;16:1637-1643.

Figure legends

Figure 1

Hematoxylin and eosin staining of hypothalamic tissue from traumatic brain injury patients reveals a spectrum of findings (arrows) including acute hemorrhage without organization (A), microglial nodules composed of lymphocytes and microglia (B), axonal swellings (C), and rarefaction of neuropil with macrophages (D).

Figure 2

Immunostaining for GFAP reveals more pronounced gliosis in TBI patients than in controls (A). The density of GFAP-immunoreactive soma is greatest near the wall of the third ventricle and basal meninges, especially in TBI patients. To quantify gliosis in TBI patients and controls, we stereotactically counted GFAP immunoreactive soma within a 3x3mm box, divided into 10 layers of 300µm width. In each layer, we determined the mean cell density by counting the number of astrocytes in 10 randomly distributed counting boxes of 100x100µm. We measured this GFAP gradient from the medial wall of the third ventricle (green box) and from the ventral surface of the hypothalamus (blue box), in one section from the anterior hypothalamus and one from the posterior hypothalamus of each subject (B). *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

Figure 3

Photomicrographs illustrate the soma, proximal dendrites, and axons of immunolabeled HDC, MCH and orexin A neurons in TBI patients and control subjects. Compared to control subjects, TBI patients had 41% fewer histaminergic TMN neurons, 29% fewer MCH neurons, and 21% fewer orexin A neurons; *** $p < 0.001$, ** $p = 0.01$.

Figure 4

Rostro-caudal distributions of histaminergic TMN neurons, MCH neurons and orexin A neurons in TBI patients and controls. TBI patients have the greatest loss of histaminergic neurons in the posterior half of the TMN. Loss of MCH neurons also tends to be higher in the posterior half of the MCH field ($37.6 \pm 35.6\%$ vs. $21.4 \pm 24.4\%$, $p=0.12$), while no rostro-caudal difference is observed in orexin A neurons ($10.4 \pm 47.6\%$ vs. $22.6 \pm 21.7\%$, $p=0.28$).

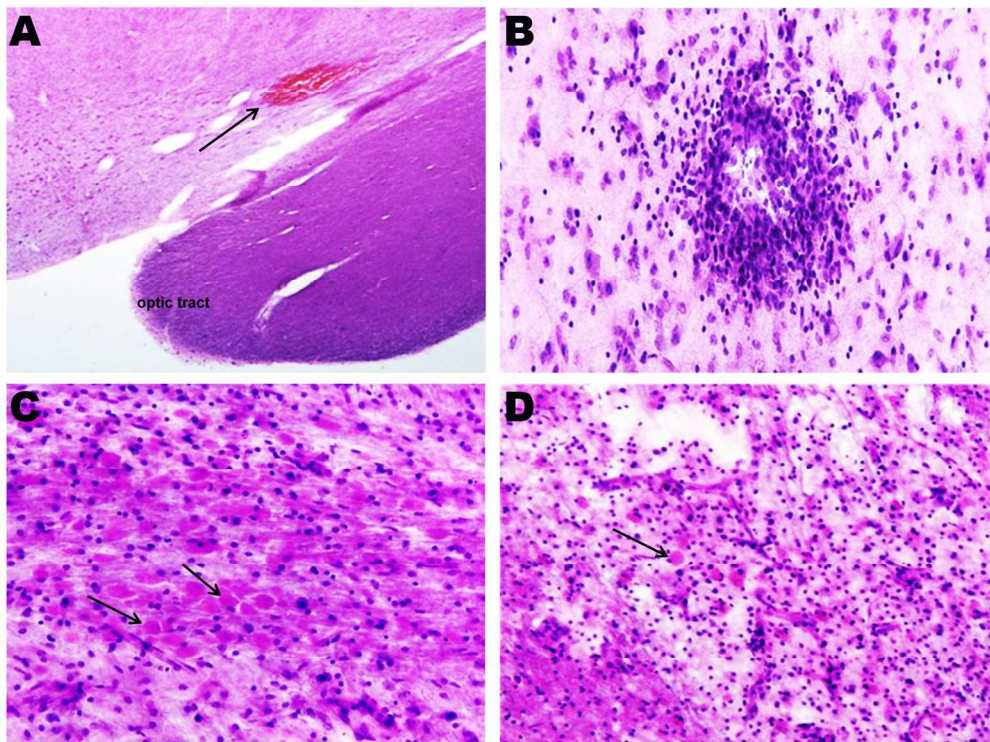


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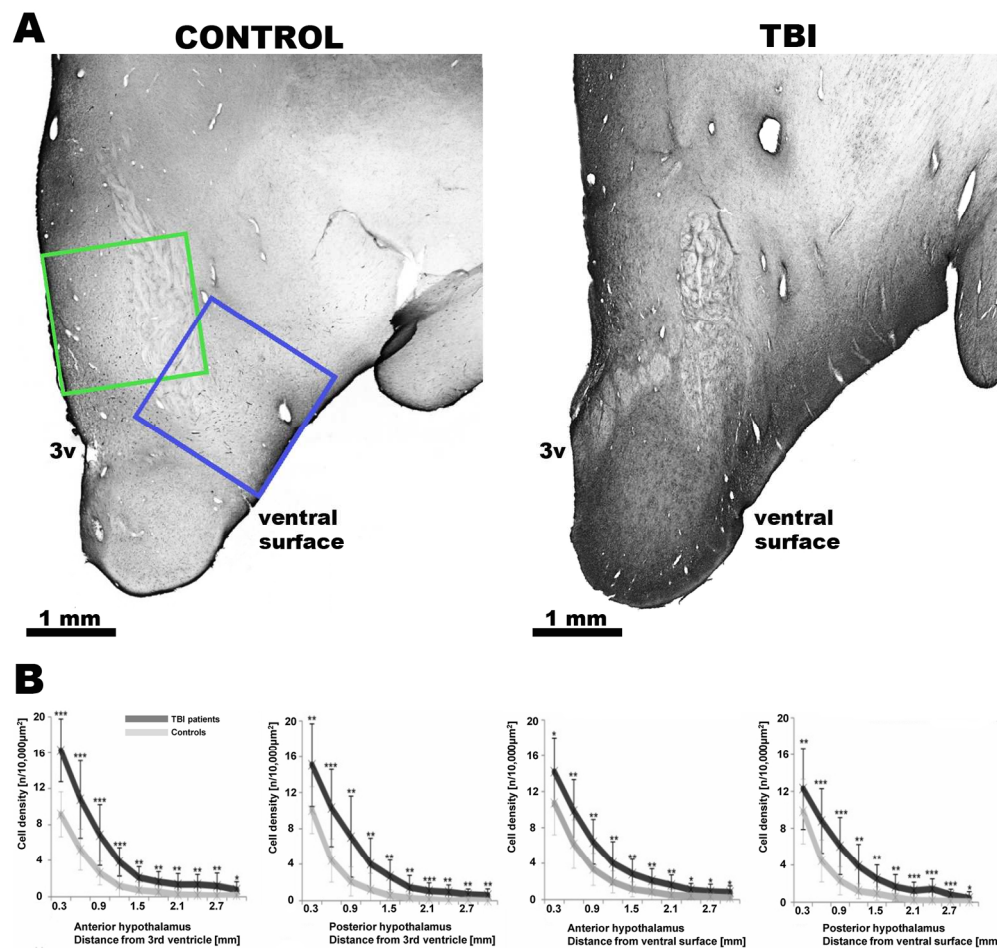


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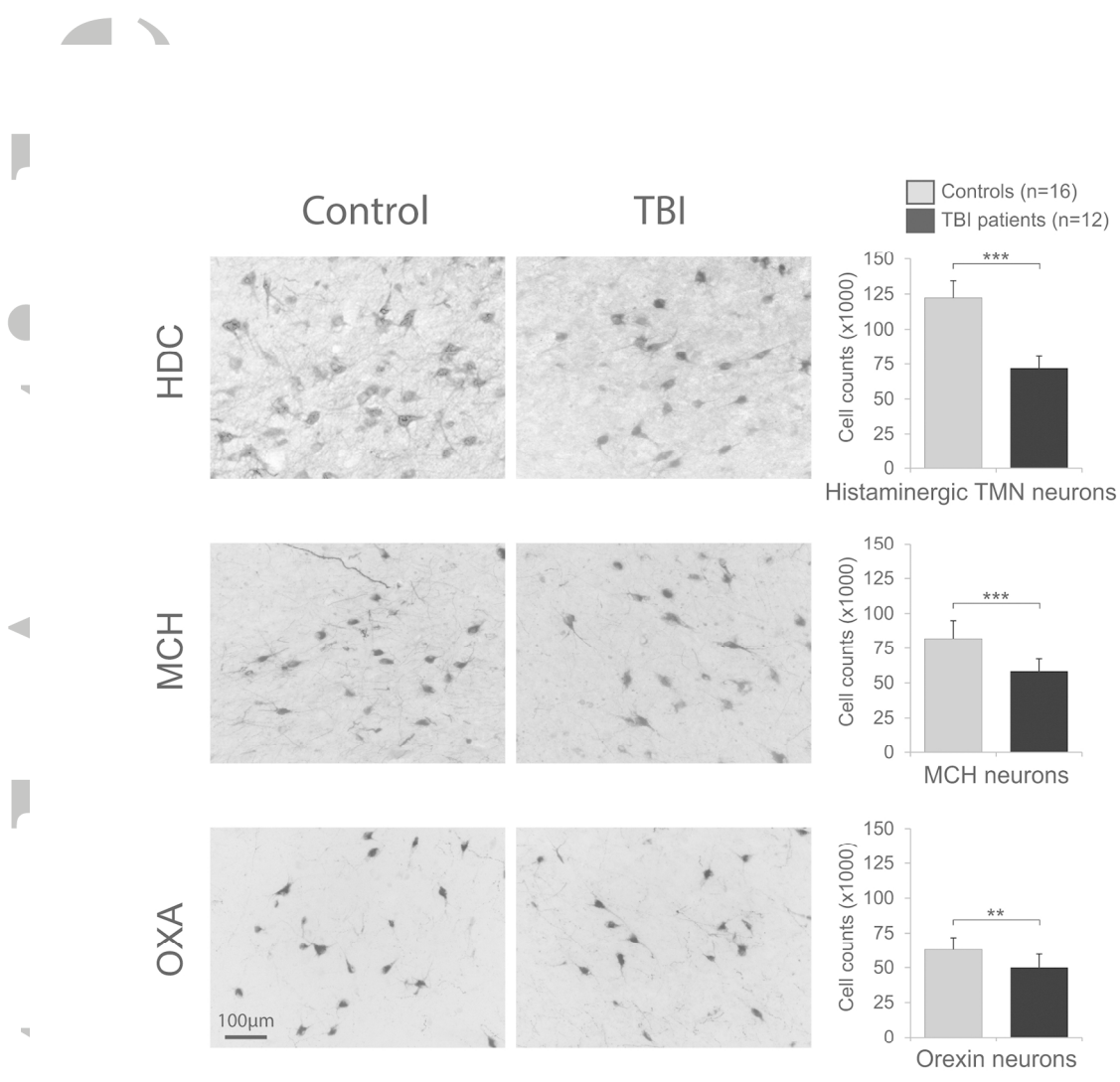


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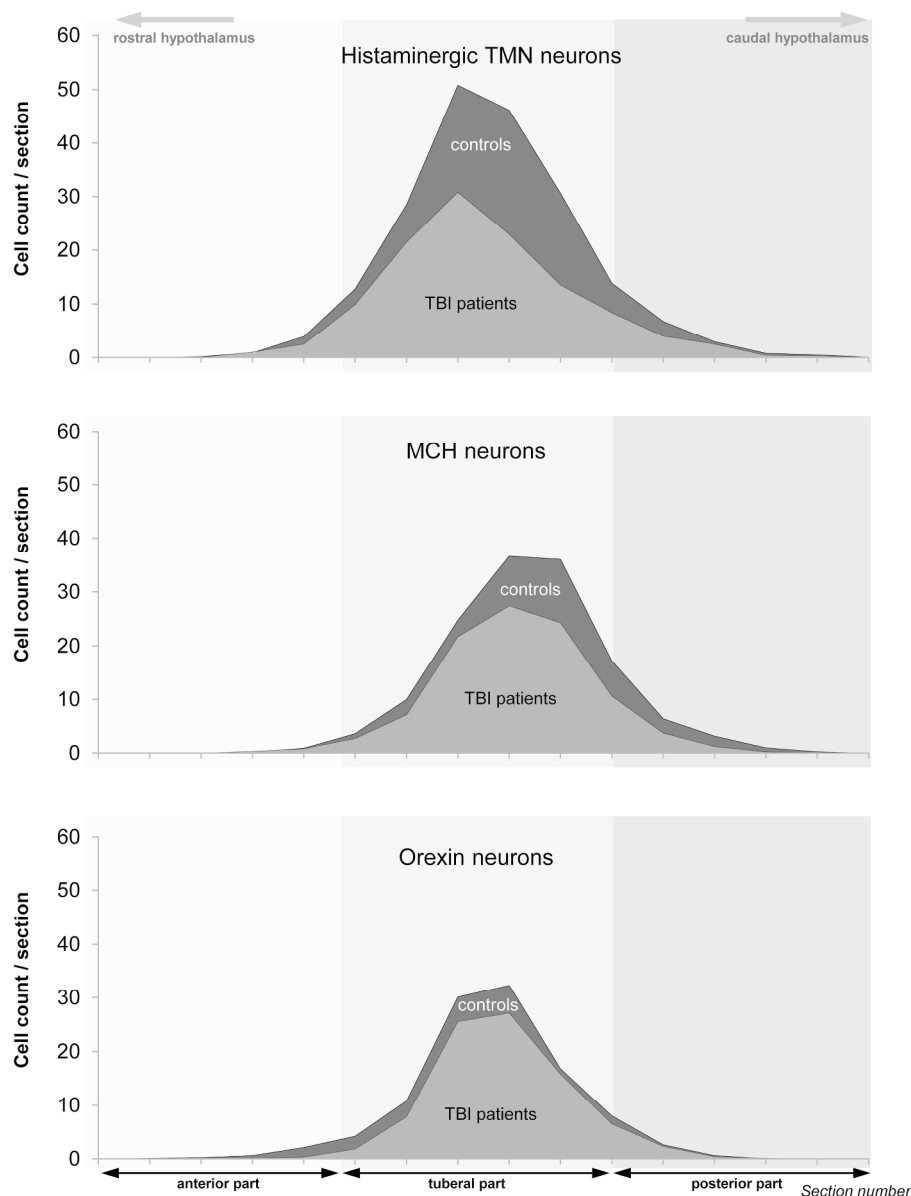


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